Localized Lymphoid Tissues and Plasma Cells in Paraocular and Paranasal Organ Systems in Chickens

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THE DOMESTIC CHICKEN has been the chief experimental animal for the study of upper respiratory virus infections in this laboratory for over a decade. Consistent finding of lymphocytic invasions and germinal centers in the lacrimal ducts of most controls prompted histologic examination of a flock known to be free of antibodies to lymphomatosis [1] and a flock which had been raised in germfree conditions. Both flocks showed similar lesions, again exclusively in the lacrimal ducts, implying some association with the eye. Sections through the orbital area disclosed, in each category of chickens, large populations of plasma cells in the glands of Harder, and a series of invasive small-lymphocyte nodules in the ducts of these glands. Further, a population of plasma cells was often found intruding among the epithelial cells lining the duct system of a pair of large compound serous glands which discharge into the nasal vestibule—the lateral nasal glands. These consistent, localized, different types of lymphocyte expression in chickens led to a sample survey of other bird species, both domestic and wild.

Most avian species, including chickens, entirely lack cervical lymph nodes [2,3] but have a system of lymphoid nodules normally associated with lymph vessels.[4,5] Lucas and his associates have studied avian lymphoid tissues in particular reference to lymphomatosis and have proposed that all invasive loci should be considered abnormal, or "ectopic." [5,6] Biggs [7] found the nodules normally associated with lymph vessels ("mural" nodules) similar in development and structure to the cervical nodes of the species which have true nodes, and differentially characterized mural and ectopic types.

Recent work in several laboratories suggests functional separation of chicken lymphoid tissues into two interrelated systems: a small-lympho-

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cyte system primarily mediated by the thymus and concerned with cellular immunity, and the germinal center and plasma cell systems primarily mediated by the bursa of Fabricius and concerned with production of immunoglobulins and specific antibodies.[8]

Materials and Methods

Histologic sections, principally through sample areas of the central and posterior nasal fossa, were made on chickens in several categories: commercial stock 18- and 20-day embryos, unhatched pipping chicks, and chicks aged 2–6 days and 1, 3, and 6 weeks. Chickens which had been tested and found free of antibodies to Rous sarcoma virus for seven generations, the "AF" flock, were sampled at 5 weeks and 1 year, as were chickens raised until the age of 6 weeks in a germfree environment. Two categories of Japanese T_2 quails, one raised in open pen or laboratory and one in germfree conditions, were also sectioned through the nasal fossa. Single specimens of 36 species of wild birds in 16 orders, acquired for a related study, were also surveyed for nasal lymphoid tissues. In the chickens and quails, at least 2, usually 3, and up to 10 specimens per category were sectioned. Fixation was 10% formalin, or 10% formalin incorporating 2% calcium acetate. Sections were stained with hematoxylin and eosin, alcian blue and periodic acid-Schiff (AB-PAS),[9] hematoxylin and PAS, or methyl green-pyronine.

For a direct view of progressive development of nodules in the area of the lacrimal duct mouth, larynx, and upper trachea at graded ages, these tissues were excised *en bloc* from stock controls fixed in formol alcohol, and stained and cleared as whole mounts, according to the method of Moe.[10]

Observations

The Lacrimal Ducts (Fig. 1, 2, and 6)

No lymphocyte foci were found in the nasolacrimal duct mucosae of 18- or 20-day embryos or unhatched pipping chicks. In one commercial flock only, all chicks had extensive lesions the third day after hatching, involving the entire mucosa of the ducts but confined to that area; they were invasive, displaced the acini, and disrupted much of the surface epithelium. Most 1-week-old chicks from other flocks had only a few small focal lesions (Fig. 3). By 3 weeks, germinal centers showed within most of the infiltrated areas; the latter sometimes coalesced (Fig. 4 and 6). The invasive process seemed to decelerate at about 3 weeks, though the numbers and sizes of lesions varied somewhat in individual chicks up to 6 weeks (Fig. 5).

Chickens from our laboratory flock of White leghorns ("AF"), which at that time had been free of antibodies to lymphomatosis for 7 generations, were killed at 5 weeks and 1 year of age. Infiltrations incorporating germinal centers were consistently found in the lacrimal ducts (Fig. 6). Equally extensive infiltrations were found in all of 9 chickens which had been raised in a germfree environment and killed at 6 weeks.

Two groups of Japanese quails, one raised in open pens and one in germfree conditions, ranging in age from 3 days to 90 weeks, were also surveyed. After the age of 3 weeks, in a total of 21 quails in the two categories, a few discrete ovoid nodules were found bilaterally in consistent sites in the mucosa of the main fossa, suggesting a natural system different from that of chickens. In the lacrimal ducts there were numbers of small invasive nodules, a total of 64% more than in the entire remainder of the mucosa. There was extensive infiltration in the lacrimal ducts of one of two open-pen adults. Germinal centers were not identified in any of the quails.

All of the adult domestic and wild species had pairs of loosely constructed faucial "tonsils." All chickens past the age of 1 week had lymphoid-tissue foci in the dorsal nasopharynx, progressively developing nodules in the larynx (Fig. 7 and 8), and by 12 weeks, nodules in the trachea (Fig. 9). Nearly all of the wild birds had a few minor lesions, some with germinal centers, in random sites in the respiratory mucosa. These foci all seem consistent with normal host responses to environmental stimuli.[6] However, nearly all adult control chickens had large germinal centers associated with the lacrimal duct and lateral nasal gland duct systems, so large that they filled all of the space normally occupied by areolar tissues.

There were no lymphocyte foci in the salivary glands of any domestic or wild bird species.

The Harderian (Nictitating Membrane) Glands (Fig. 2)

These glands lubricate the nictitating membranes. In phylogenetic history they have become greatly developed in many mammals and birds, but in the ascending primate line have progressively decreased, and are transitory in the human embryo.[11] In chickens they are large, multilobular, and tubulo-acinar. The mucous secretion stains pure alcian blue in the AB-PAS procedure with AB at pH 1.[9]

In the intralobular connective tissues of these glands, in unhatched pipping chicks and 1-week-old chicks, there was a population of heterophils in concentrations markedly greater than elsewhere in any tissues in the head except for the bone marrow. By the age of 2 weeks, and up to 6 weeks, these intralobular tissues were conspicuously swollen with numbers of plasma cells and heterophils (Fig. 10). Large populations of plasma cells were found in the same tissues of the Harderian glands of AF and germfree chickens, open flock and germfree (Fig. 11) quails, and five species of wild birds: a grebe, *Podiceps ruficollis*, a cuckoo, *Centropus sinensis*, a sunbird, *Nectarinia zeylonica*, all from

West Bengal; and two species of rail, *Rallus limicola* and *R. longirostris*, from Maryland.

The Harderian Gland Ducts (Fig. 2)

The ducts, which convey gland fluids to the nictitating membranes, are lined normally with villi invested with a cuboid and goblet-cell epithelium. In all ages and categories of chickens from the age of 1 week on, and in two adult quails sectioned through the ducts, the normal tissues of the villi had been replaced by lymphoid infiltrations along the entire course of the ducts and the adjoining part of the nictitating membrane. In sections made at different time sequences, it was seen that these began as light invasions which proliferated until the villi were converted into club-shaped lymphoid nodules (Fig. 12), and the normal epithelium was replaced by a flat, thin squamous sheet.

The Lateral Nasal Gland Ducts

The lateral nasal glands (Fig. 1) of land birds are probably the chief source of the physiological vapor needed for olfaction and respiration.[12] In nearly all vertebrates, including chickens, their serous secretion is discharged into the nasal vestibules in the form of droplets which are evidently atomized at each inspired breath. The collecting ductules and main ducts of the gland are lined with a single layer of columnar epithelial cells. In most stock and AF chickens, there was a row of plasma cells intruding between the bases of these cells. Extremely rare in the 9 germfree chickens in our sample, the population of plasma cells varied markedly in stock individuals and sometimes in flocks; for example, 100% of ten 2-week-old chicks in one flock had the plasma cells in significant numbers (Fig. 13). Plasma cells were not seen in any of the quails or wild birds in this site.

Harderian secretions either spill out of the eyes or are discharged via the lacrimal ducts into the mouth, to be swallowed or expectorated. Lateral nasal gland secretions discharge into the nasal vestibule, thence to be inhaled or to crystallize at the rim of the nares. All of these fluids could be dispensed into drinking or feeding troughs, and all would be dispersed as droplets in sneezing.

Discussion

The very location of intranasal lymphoid nodules, projecting into the lumen in the path of the foreign debris and infectious agents carried by the mucociliary sheet, suggests their natural function. The fact that foci of lymphocyte-line cells appear as different expressions of activity in particular sites in the respiratory and lacrimal systems raises ques-

tions of normal (ontogenic) or abnormal (pathologic) induction that cannot be answered until much more is known of the behavior of the most important respiratory agents. Even the exhaustive studies of Lucas *et al.*[5,6] on lymphoid foci in chickens, in which it was not possible differentially to define normal and abnormal, do not include upper respiratory or ocular gland tissues. Nor has Marek's disease, now clearly separable from the leukosis complex, been studied in the upper respiratory tract. The presence of plasma cells in the nasal gland ducts suggests secretory antibody.

At present, then, one cannot distinguish between the normal development of locally functioning systems capable of responding to infectious agents and cell types brought to the area in response to one or more specific agents; and functionally the distinction between normal and abnormal lymphocyte foci may be academic.

In respect to the four tissue systems which respond in different ways to environmentally introduced agents:

1. The lacrimal duct lesions were found only in domestic gallinaceous birds, not in any wild species. The lesions in apparently healthy controls are indistinguishable from those found in the nasal mucosae of chickens known to be infected with mycoplasma and laryngotracheitis virus.[13] The massive infiltrations occasionally found at 3 days suggest the possibility of egg transmission. Mycoplasma, lymphomatosis, Marek's disease, and other highly contagious chicken pathogens produce extensive lymphocyte response in nature; the first two are transmissible through the egg, but the point has not been established for Marek's disease. There is a great local and periodic variation in the prevalence of these and other respiratory agents; a survey in India [14] showed that 90% of a group of apparently healthy chickens had antibodies against one or more of four agents tested, and a survey in Wisconsin recorded an increase in the incidence of Marek's disease from 2.7% to 84.3% within 2 years.[15]

2. The Harderian gland tissues of individual birds of all wild and domestic species sampled so far have had abnormal numbers of either heterophils or plasma cells or both. The concentrations of heterophils in this specific site in unhatched pipping chicks implies an *in ovo* stimulus, and the often extraordinary numbers of Harderian plasma cells in germfree, AF, and wild species suggest a prominent role for this organ in the avian immune system. While mycoplasma has been isolated from commercially grown bobwhite quails,[16] there are no satisfactory data on wild birds as reservoirs of this or any other respiratory agent.

3. The great numbers of prominent nodules in the Harderian ducts

could represent either an initial phase of Harderian response or an unrelated local response. In human nasal infections it has been demonstrated that local immunologic systems may produce distinctive types of antibody response [17,18] and that neutralizing activity associated with a particular immunoglobulin may be found in both lacrimal and nasal secretions.[19]

4. The most variable focal response was the plasma cell population in the lateral nasal gland ducts. While, in the present study, it was manifest only in chickens, the importance of the serous nasal glands in animal models has been generally overlooked in respiratory pathobiology. Not only are they probably the main source of the physiological vapor essential for respiration and olfaction, but their secretion is both inhaled and shed in the nasal vestibule. The human counterparts of these compound glands are the serous acini in the mixed alveoli of the anterior nasal glands,[20] which are the first nasal glands to form and the first to secrete in the human embryo.[21] The immunofluorescence studies of Rossen and his associates indicate that the serous nasal acini in humans may selectively produce immunoglobulin A.[22]

While the overwhelming dependence of chicken lymphoid organs on the developing thymus and bursa of Fabricius has been amply demonstrated,[8] the oculonasal gland systems have not been included in surveys of affected organ systems. Since there is now good evidence that both lymphoid cells and granulocytes originate from myeloid stem cells,[23,24] the possibility that organs other than "central lymphoid organs" may serve as ancillary or local lymphopoietic systems should not be overlooked, especially in an animal that is easily thymectomized and bursectomized.

Summary

Invasive lymphocyte populations have been consistently found in four organized tissue systems in apparently healthy commercial stock chickens: in the lacrimal ducts, the Harderian glands, the ducts of those glands, and the duct system of the lateral nasal glands.

The lacrimal duct lesions consisted of small-lymphocyte infiltrations and germinal centers; the Harderian glands were infiltrated with large populations of plasma cells; the gland ducts by a series of invasive small-lymphocyte nodules; and the lateral nasal gland ducts by plasma cells which intruded among the epithelial lining cells.

The lateral nasal gland invasion was confined to chickens, the lacrimal duct lesions to chickens and domestic quails. The Harderian population, however, was found in wild birds, as well as in germfree chickens and quails, and chickens free of antibodies to lymphomatosis virus.

Each of these tissue systems is associated with an area of entry into, and shedding from, the upper respiratory tract. Each may be capable of local immune response to environmental stimuli.

Addendum

After this report was submitted, 3 chickens which had been hatched and raised for 5 weeks in germfree conditions from eggs of the ninth generation of our AF flock were received from the Lobund Laboratory and were sectioned and stained. All show lymphocyte infiltration and germinal centers in the lacrimal ducts, a significant plasma cell population in the subepithelium of the interlobular lumen in the Harderian gland, invasive small lymphocytes and some germinal centers in the ducts of those glands, and plasma cells along the base of the ducts and ductules of the lateral nasal glands.

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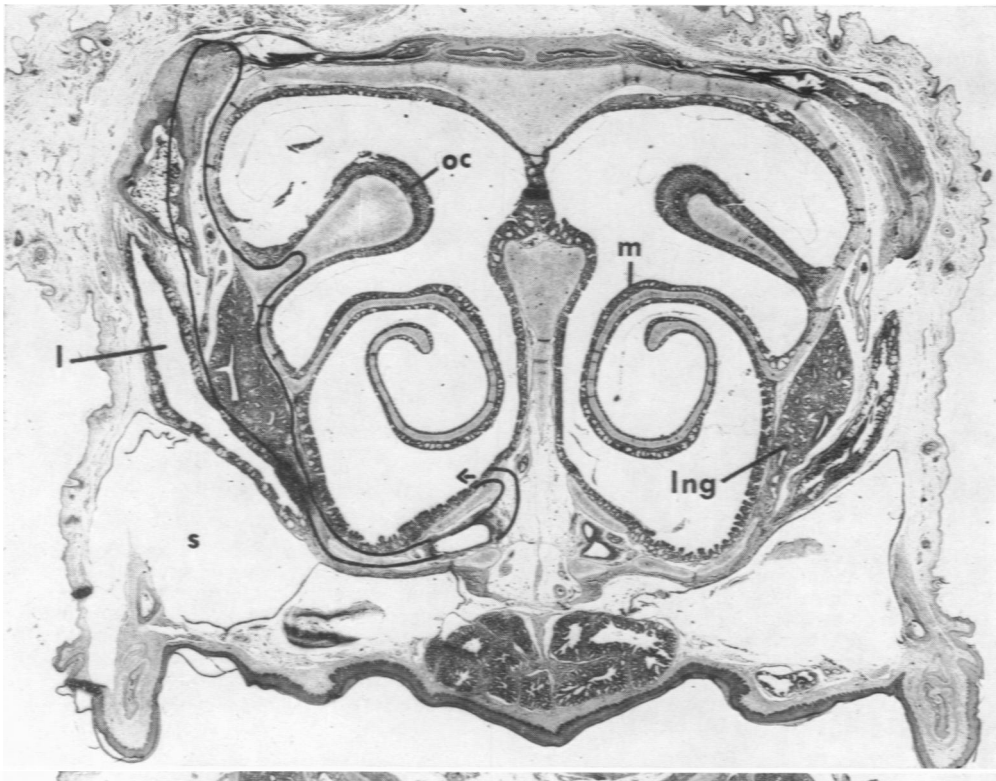
All germfree quails were obtained from the late Dr. James A. Reyniers of the Germfree Research Center, Tampa, Fla., and all germfree chickens from Dr. Morris Pollard of the Lobund Laboratory, Notre Dame, Ind.

Legends for Figures

All histologic sections were stained with hematoxylin and eosin; all whole-mount preparations, with periodic acid-Schiff stain (PAS).

Fig. 1. Section through midnasal fossa of chicken. Full extent of lateral nasal glands and duct indicated by outline on left side. × 40.

Fig. 2. Posterior nasal fossa of same chicken. Full extent of Harderian glands and duct indicated by outlining. × 40.



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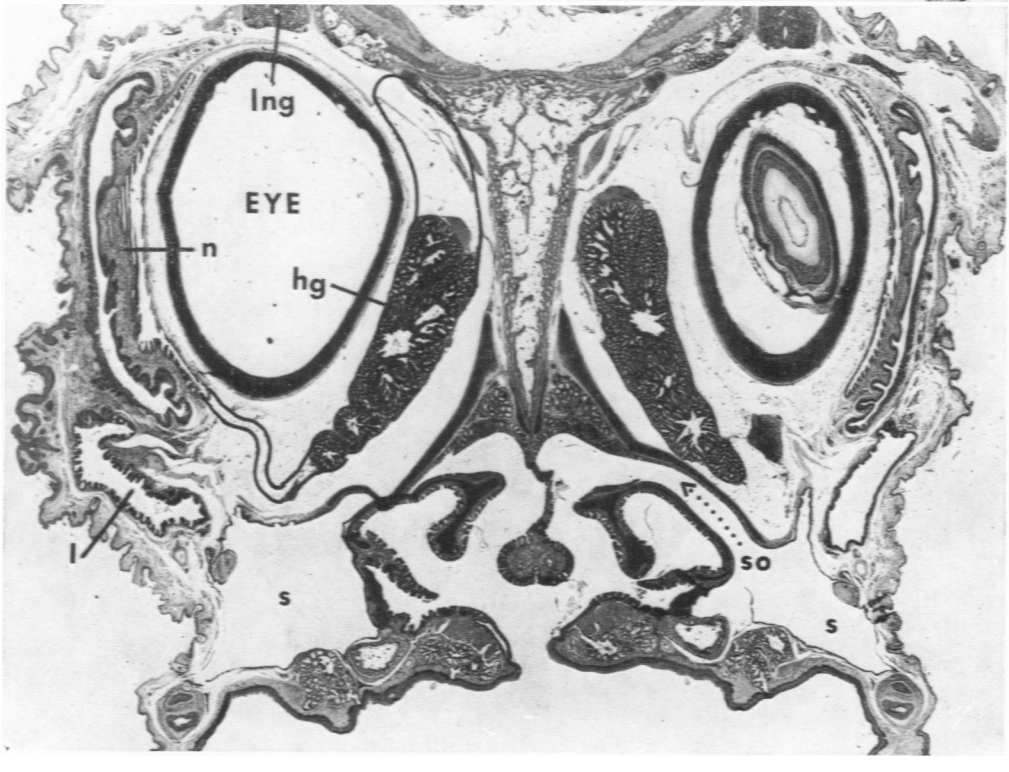

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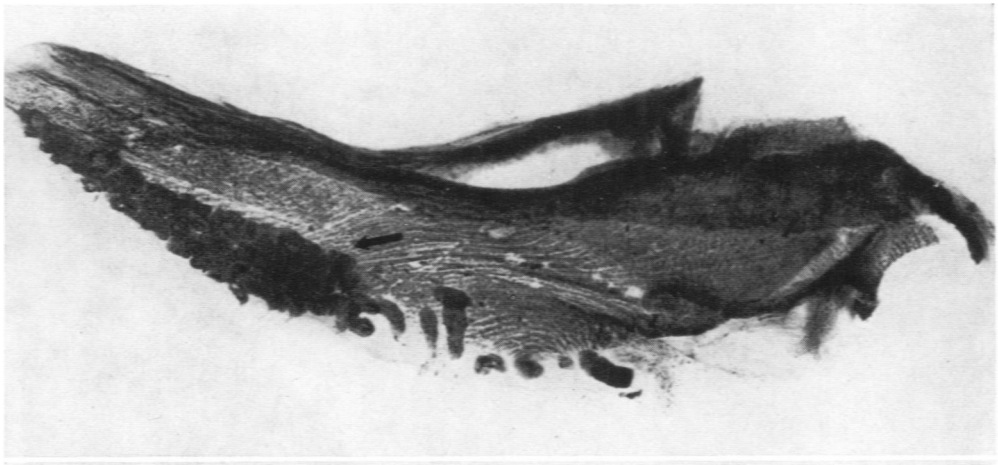
Fig. 3–5. Whole-mount dissections of lacrimal duct mouth area of 1-, 3-, and 6-week-old chickens, respectively. When mounts are stained with PAS and cleared in anise oil, PAS stains only the mucous glands, and acini show as patterned lines. Large unstained areas and discrete ovals proved microscopically to be invasive lymphoid tissues and lymphoid nodules, which do not stain with PAS. Thus an overview of the total acinar displacement is obtained without the necessity of serial reconstruction. Clear rows between acini show microscopically as ciliated cells, and arrows indicate direction of mucociliary flow as demonstrated in freshly killed chicks with India ink.[13] **Fig. 3,** × 12; **Fig. 4,** × 9; **Fig. 5,** × 8.



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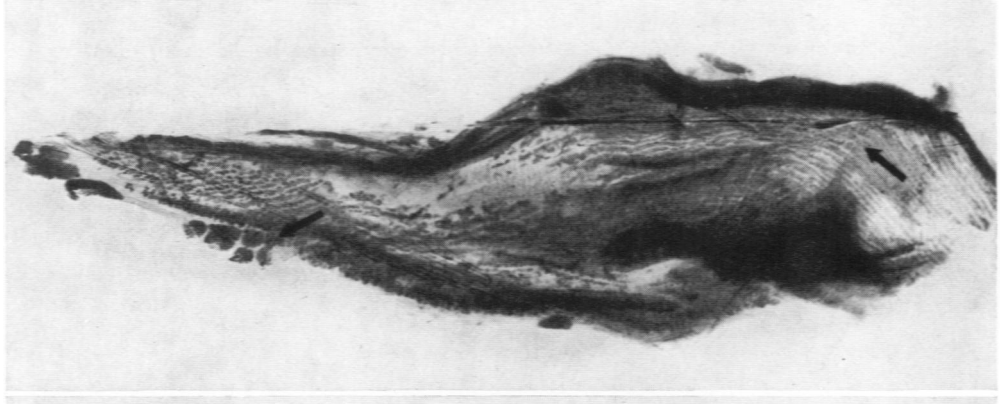

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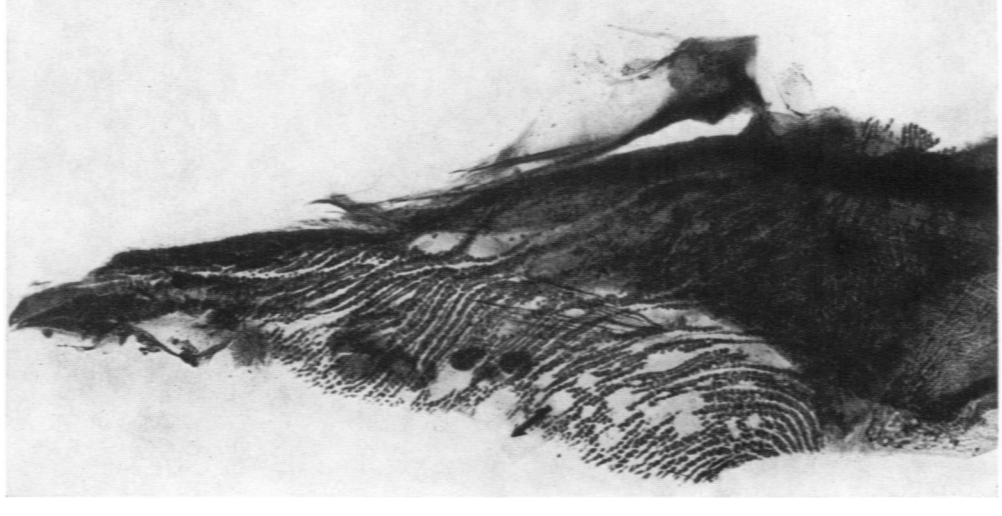

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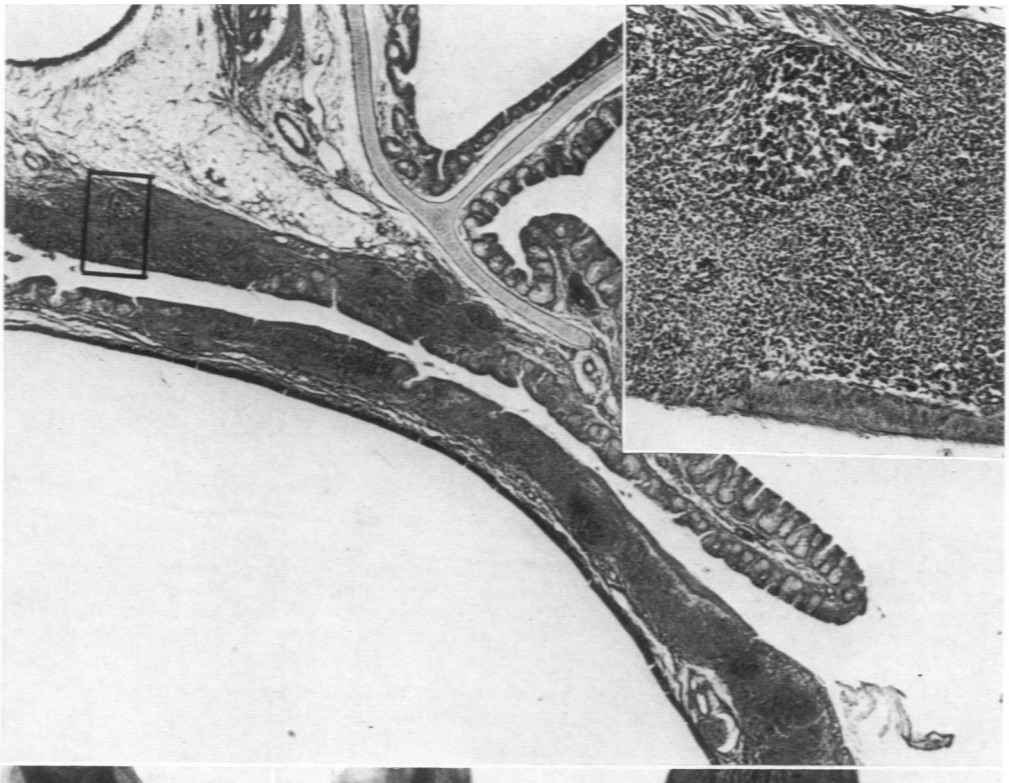
Fig. 6. Portion of lacrimal duct of 5-week-old lymphomatosis-free chicken, showing germinal centers and lymphocyte infiltrations which have coalesced. × 15. **Inset.** Enlargement of one germinal center. × 150.

Fig. 7. Whole-mount dissection of ventral half of larynx and upper trachea of 1-week-old chick, PAS stained, and cleared in anise oil. As in Fig. 3–5, lymphoid nodules do not stain, unstained rows of ciliated cells show between stained rows of acini, and arrows show direction of mucociliary flow. × 12.

Fig. 8. Same area in 3-week-old chick. × 8.

Fig. 9. Same area in 12-week-old chick. By this age, lymphoid nodules have appeared in the larynx. Smudge in the larynx is mucus. × 6.



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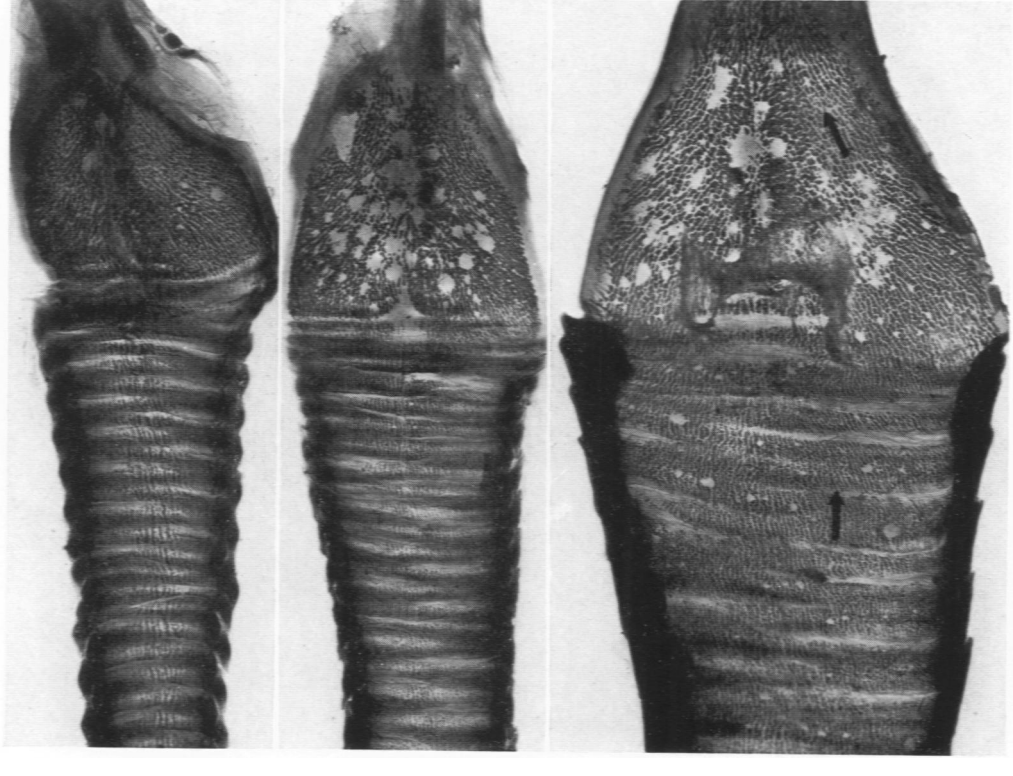

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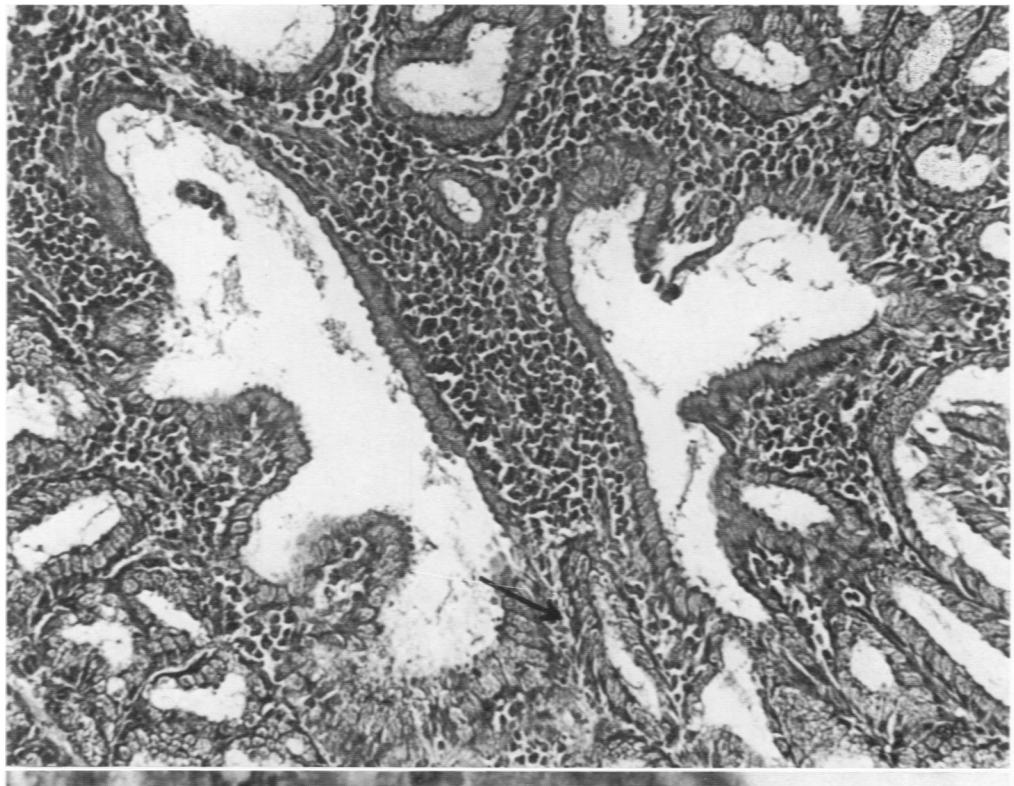
Fig. 10. Portion of an interlobular lumen in Harderian gland of 6-week-old stock chicken which was raised under germfree conditions, showing extent of infiltration of subepithelial stroma by a population of cells, most of which are seen in higher power to be plasma cells (Fig. 11). Arrow points to a small area of uninvaded stroma. × 200.

Fig. 11. Interlobular lumen of Harderian gland of 90-week-old germfree Japanese T_2 quail, showing plasma cells in stroma of subepithelium. × 1000.



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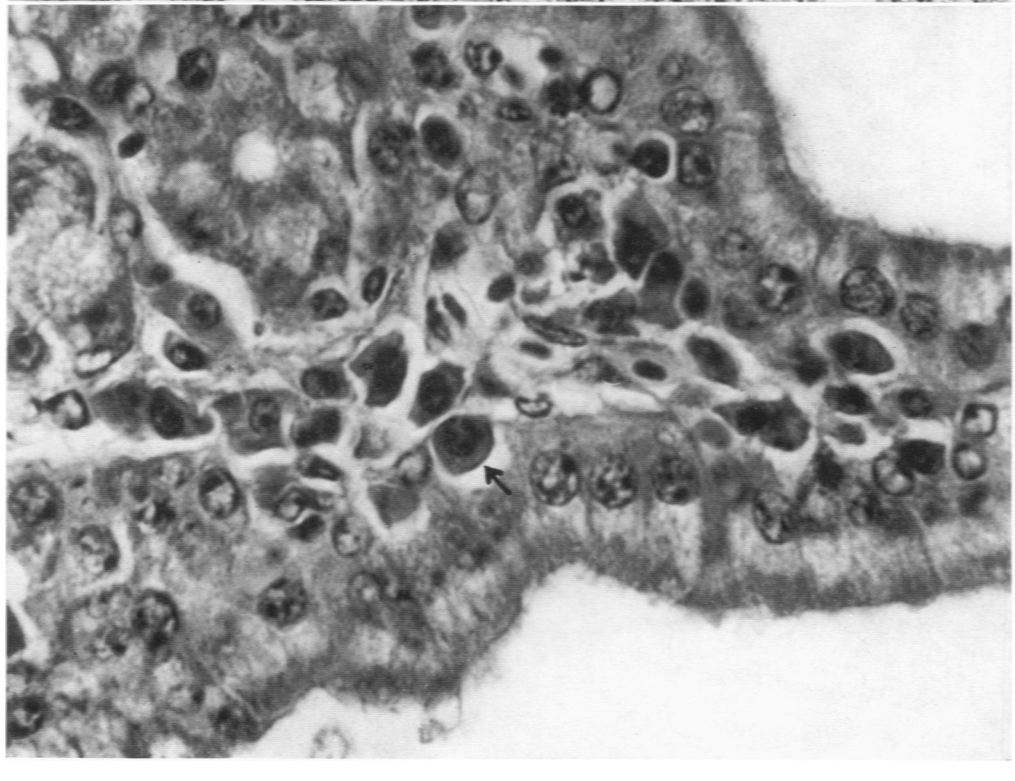

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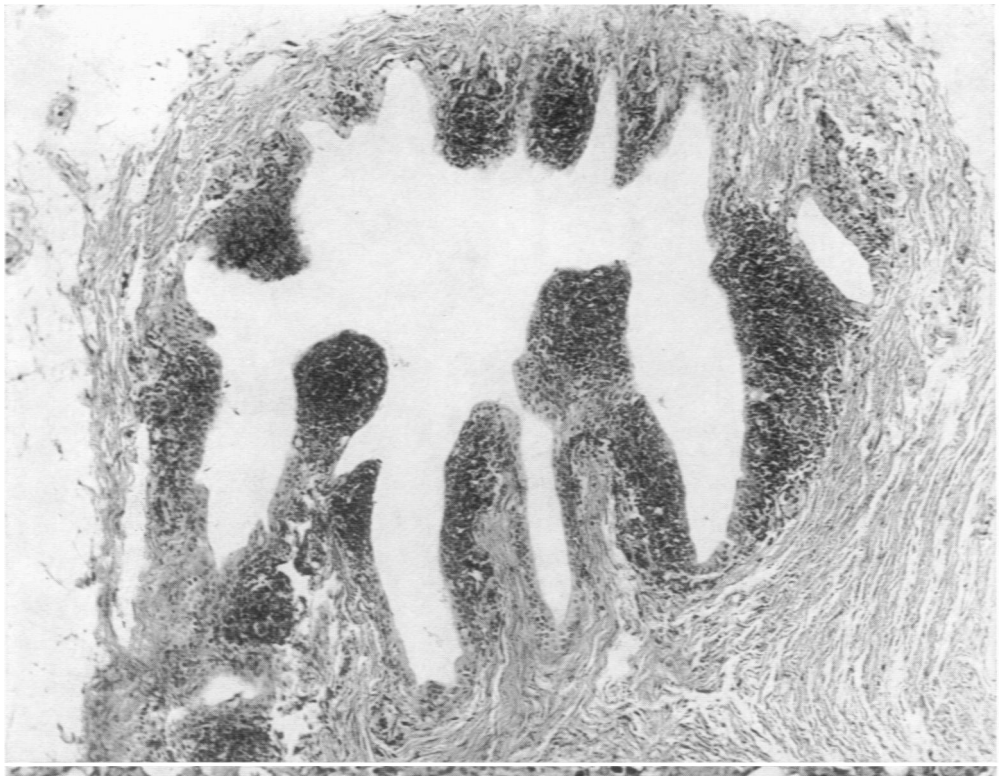
Fig. 12. Section through main duct of gland of Harder of 6-week-old chicken raised under germfree conditions, showing epithelial villi invaded by lymphoid cells. × 100.

Fig. 13. Main duct *(d)* and ductule *(du)* of lateral nasal gland of 2-week-old stock chick, showing numbers and distribution of plasma cells—dark cells surrounded by a clear space *(arrows)*—aligned along base of duct epithelium. × 200.



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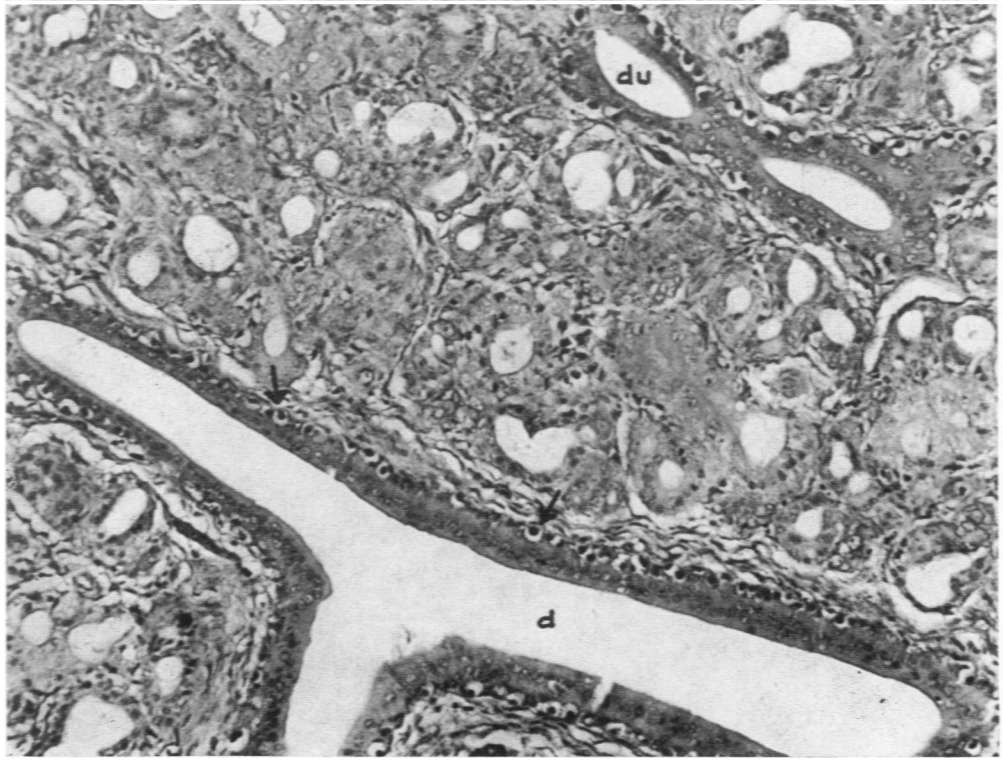

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